```
  1 ATGGCGCCAC CACCAGCTAG AGTACATCTA GGTGCGTTCC TGGCAGTGAC
    TACCGCGGTG GTGGTCGATC TCATGTAGAT CCACGCAAGG ACCGTCACTG
  1 MetAlaProP roProAlaAr gValHisLeu GlyAlaPheL euAlaValTh

 51 TCCGAATCCC GGGAGCGCAG CGAGTGGGAC AGAGGCAGCC GCGGCCACAC
    AGGCTTAGGG CCCTCGCGTC GCTCACCCTG TCTCCGTCGG CGCCGGTGTG
    rProAsnPro GlySerAlaA laSerGlyTh rGluAlaAla AlaAlaThrPro

101 CCAGCAAAGT GTGGGGCTCT TCCGCGGGGA GGATTGAACC ACGAGGCGGG
    GGTCGTTTCA CACCCCGAGA AGGCGCCCCT CCTAACTTGG TGCTCCGCCC
 35    SerLysVa lTrpGlySer SerAlaGlyA rgIleGluPr oArgGlyGly

151 GGCCGAGGAG CGCTCCCTAC CTCCATGGGA CAGCACGGAC CCAGTGCCCG
    CCGGCTCCTC GCGAGGGATG GAGGTACCCT GTCGTGCCTG GGTCACGGGC
    GlyArgGlyA laLeuProTh rSerMetGly GlnHisGlyP roSerAlaArg

201 GGCCCGGGCA GGGCGCGCCC CAGGACCCAG GCCGGCGCGG GAAGCCAGCC
    CCGGGCCCGT CCCGCGCGGG GTCCTGGGTC CGGCCGCGCC CTTCGGTCGG
 68  AlaArgAla GlyArgAlaP roGlyProAr gProAlaArg GluAlaSerP

251 CTCGGCTCCG GGTCCACAAG ACCTTCAAGT TTGTCGTCGT CGGGGTCCTG
    GAGCCGAGGC CCAGGTGTTC TGGAAGTTCA AACAGCAGCA GCCCCAGGAC
    roArgLeuAr gValHisLys ThrPheLysP heValValVa lGlyValLeu

301 CTGCAGGTCG TACCTAGCTC AGCTGCAACC ATGATCAATC AATTGGCACA
    GACGTCCAGC ATGGATCGAG TCGACGTTGG TAGTTTGAAG TACTAGTTAG
101 LeuGlnValV alProSerSe rAlaAlaThr IleLysLeuH isAspGlnSe

351 AATTGGCACA CAGCAATGGG AACATAGCCC TTTGGGAGAG TTGTGTCCAC
    TTAACCGTGT GTCGTTACCC TTGTATCGGG AAACCCTCTC AACACAGGTG
    rIleGlyThr GlnGlnTrpG luHisSerPr oLeuGlyGlu LeuCysProPro

401 CAGGATCTCA TAGATCAGAA CGTCCTGGAG CCTGTAACCG GTGCACAGAG
    GTCCTAGAGT ATCTAGTCTT GCAGGACCTC GGACATTGGC CACGTGTCTC
135    GlySerHi sArgSerGlu ArgProGlyA laCysAsnAr gCysThrGlu

451 GGTGTGGGTT ACACCAATGC TTCCAACAAT TTGTTTGCTT GCCTCCCATG
    CCACACCCAA TGTGGTTACG AAGGTTGTTA AACAAACGAA CGGAGGGTAC
    GlyValGlyT yrThrAsnAl aSerAsnAsn LeuPheAlaC ysLeuProCys

501 TACAGCTTGT AAATCAGATG AAGAAGAGAG AAGTCCCTGC ACCACGACCA
    ATGTCGAACA TTTAGTCTAC TTCTTCTCTC TTCAGGGACG TGGTGCTGGT
168  ThrAlaCys LysSerAspG luGluGluAr gSerProCys ThrThrThrA

551 GGAACACAGC ATGTCAGTGC AAACCAGGAA CTTTCCGGAA TGACAATTCT
    CCTTGTGTCG TACAGTCACG TTTGGTCCTT GAAAGGCCTT ACTGTTAAGA
    rgAsnThrAl aCysGlnCys LysProGlyT hrPheArgAs nAspAsnSer

601 GCTGAGATGT GCCGGAAGTG CAGCACAGGG TGCCCCAGAG GGATGGTCAA
    CGACTCTACA CGGCCTTCAC GTCGTGTCCC ACGGGGTCTC CCTACCAGTT
201 AlaGluMetC ysArgLysCy sSerThrGly CysProArgG lyMetValLy

651 GGTCAAGGAT TGTACGCCCT GGAGTGACAT CGAGTGTGTC CACAAAGAAT
    CCAGTTCCTA ACATGCGGGA CCTCACTGTA GCTCACACAG GTGTTTCTTA
    sValLysAsp CysThrProT rpSerAspIl eGluCysVal HisLysGluSer
```

FIG._1A

```
 701 CAGGCAATGG ACATAATATA TGGGTGATTT TGGTTGTGAC TTTGGTTGTT
     GTCCGTTACC TGTATTATAT ACCCACTAAA ACCAACACTG AAACCAACAA
 235   GlyAsnGl yHisAsnIle TrpValIleL euValValTh rLeuValVal

 751 CCGTTGCTGT TGGTGGCTGT GCTGATTGTC TGTTGTTGCA TCGGCTCAGG
     GGCAACGACA ACCACCGACA CGACTAACAG ACAACAACGT AGCCGAGTCC
     ProLeuLeuL euValAlaVa lLeuIleVal CysCysCysI leGlySerGly

 801 TTGTGGAGGG GACCCCAAGT GCATGGACAG GGTGTGTTTC TGGCGCTTGG
     AACACCTCCC CTGGGGTTCA CGTACCTGTC CCACACAAAG ACCGCGAACC
 268  CysGlyGly AspProLysC ysMetAspAr gValCysPhe TrpArgLeuG

 851 GTCTCCTACG AGGGCCTGGG GCTGAGGACA ATGCTCACAA CGAGATTCTG
     CAGAGGATGC TCCCGGACCC CGACTCCTGT TACGAGTGTT GCTCTAAGAC
     lyLeuLeuAr gGlyProGly AlaGluAspA snAlaHisAs nGluIleLeu

 901 AGCAACGCAG ACTCGCTGTC CACTTTCGTC TCTGAGCAGC AAATGGAAAG
     TCGTTGCGTC TGAGCGACAG GTGAAAGCAG AGACTCGTCG TTTACCTTTC
 301 SerAsnAlaA spSerLeuSe rThrPheVal SerGluGlnG lnMetGluSe

 951 CCAGGAGCCG GCAGATTTGA CAGGTGTCAC TGTACAGTCC CCAGGGGAGG
     GGTCCTCGGC CGTCTAAACT GTCCACATGT ACATGTCAGG GGTCCCCTCC
     rGlnGluPro AlaAspLeuT hrGlyValTh rValGlnSer ProGlyGluAla

1001 CACAGTGTCT GCTGGGACCG GCAGAAGCTG AAGGGTCTCA GAGGAGGAGG
     GTGTCACAGA CGACCCTGGC CGTCTTCGAC TTCCCAGAGT CTCCTCCTCC
 335   GlnCysLe uLeuGlyPro AlaGluAlaG luGlySerGl nArgArgArg

1051 CTGCTGGTTC CAGCAAATGG TGCTGACCCC ACTGAGACTC TGATGCTGTT
     GACGACCAAG GTCGTTTACC ACGACTGGGG TGACTCTGAG ACTACGACAA
     LeuLeuValP roAlaAsnGl yAlaAspPro ThrGluThrL euMetLeuPhe

1101 CTTTGACAAG TTTGCAAACA TCGTGCCCTT TGACTCCTGG GACCAGCTCA
     GAAACTGTTC AAACGTTTGT AGCACGGGAA ACTGAGGACC CTGGTCGAGT
 368  PheAspLys PheAlaAsnI leValProPh eAspSerTrp AspGlnLeuM

1151 TGAGGCAGCT GGACCTCACG AAAAATGAGA TCGATGTGGT CAGAGCTGGT
     ACTCCGTCGA CCTGGAGTGC TTTTTACTCT AGCTACACCA GTCTCGACCA
     etArgGlnLe uAspLeuThr LysAsnGluI leAspValVa lArgAlaGly

1201 ACAGCAGGCC CAGGGGATGC CTTGTATGCA ATGCTGATGA AATGGGTCAA
     TGTCGTCCGG GTCCCCTACG GAACATACGT TACGACTACT TTACCCAGTT
 401 ThrAlaGlyP roGlyAspAl aLeuTyrAla MetLeuMetL ysTrpValAs

1251 CAAAACTGGA CGGAACGCCT CGATCCACAC CCTGCTGGAT GCCTTGGAGA
     GTTTTGACCT GCCTTGCGGA GCTAGGTGTG GGACGACCTA CGGAACCTCT
     nLysThrGly ArgAsnAlaS erIleHisTh rLeuLeuAsp AlaLeuGluArg

1301 GGATGGAAGA GAGACATGCA AAAGAGAAGA TTCAGGACCT CTTGGTGGAC
     CCTACCTTCT CTCTGTACGT TTTCTCTTCT AAGTCCTGGA GAACCACCTG
 435   MetGluGl uArgHisAla LysGluLysI leGlnAspLe uLeuValAsp

1351 TCTGGAAAGT TCATCTACTT AGAAGATGGC ACAGGCTCTG CCGTGTCCTT
     AGACCTTTCA AGTAGATGAA TCTTCTACCG TGTCCGAGAC GGCACAGGAA
     SerGlyLysP heIleTyrLe uGluAspGly ThrGlySerA laValSerLeu

1401 GGAGTGA
     CCTCACT
 468 GluOP*
```

FIG._1B

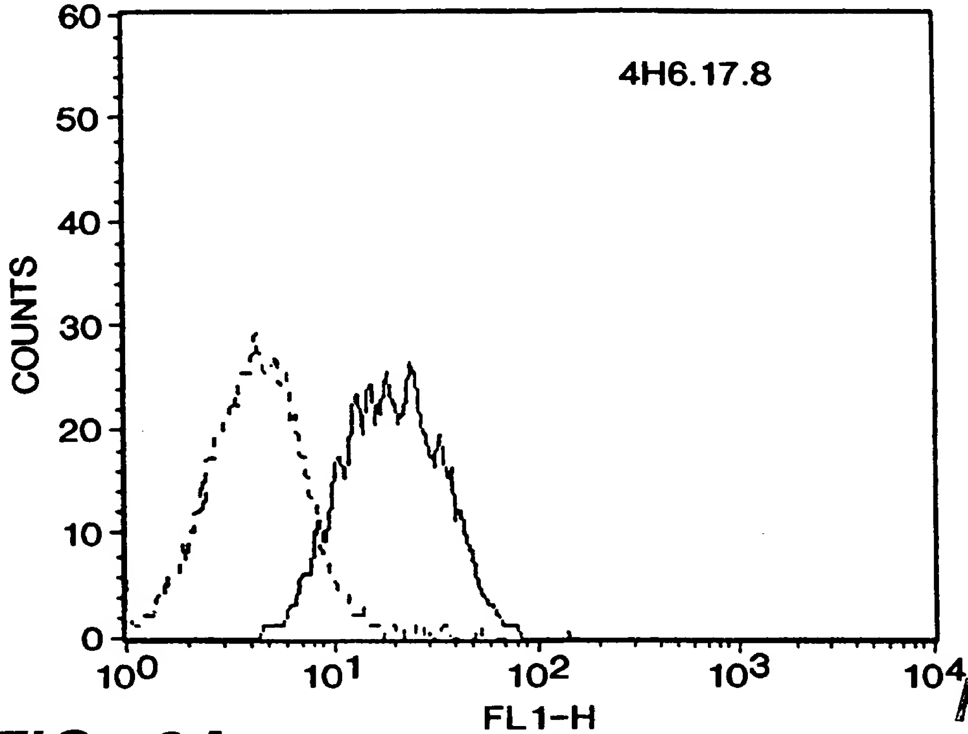

4H6.17.8
COUNTS
60
50
40
30
20
10
0
10^0
10^1
10^2
10^3
10^4
FL1-H

FIG._2A

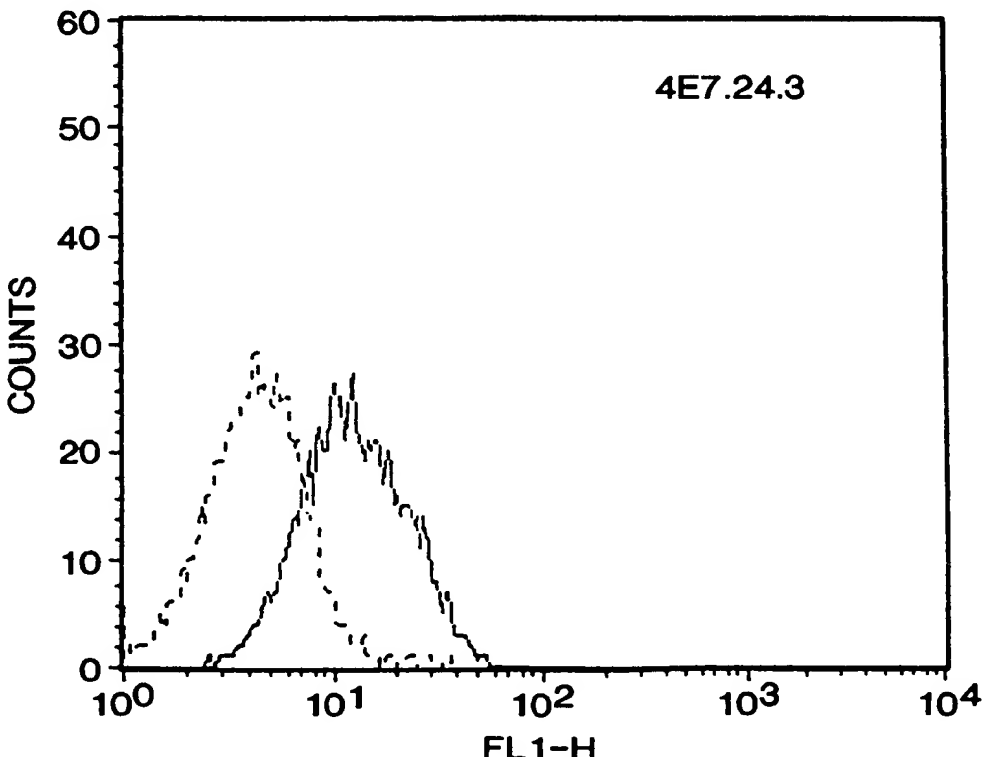

4E7.24.3
COUNTS
60
50
40
30
20
10
0
10^0
10^1
10^2
10^3
10^4
FL1-H

FIG._2B

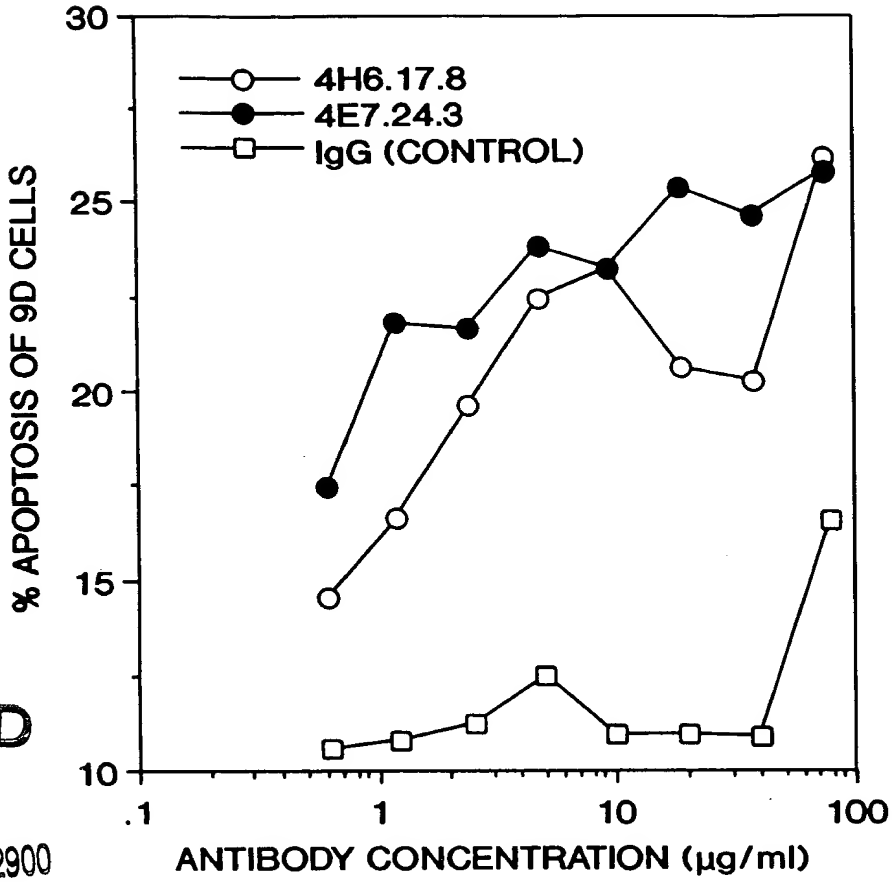


FIG._3

AFFINITIES OF Apo2Rs AND mAbs

		AFFINITY (pM)
DR4-IgG	to Apo2L	82
DR5-IgG	to Apo2L	1
mAb 4E7	to DR4-IgG	2
mAb 4H6	to DR4-IgG	5
mAb 5G11	to DR4-IgG	22
mAb 3F11	to DR5-IgG	20
mAb 3H3	to DR5-IgG	3

Affinities were determined using KinExA

FIG._7

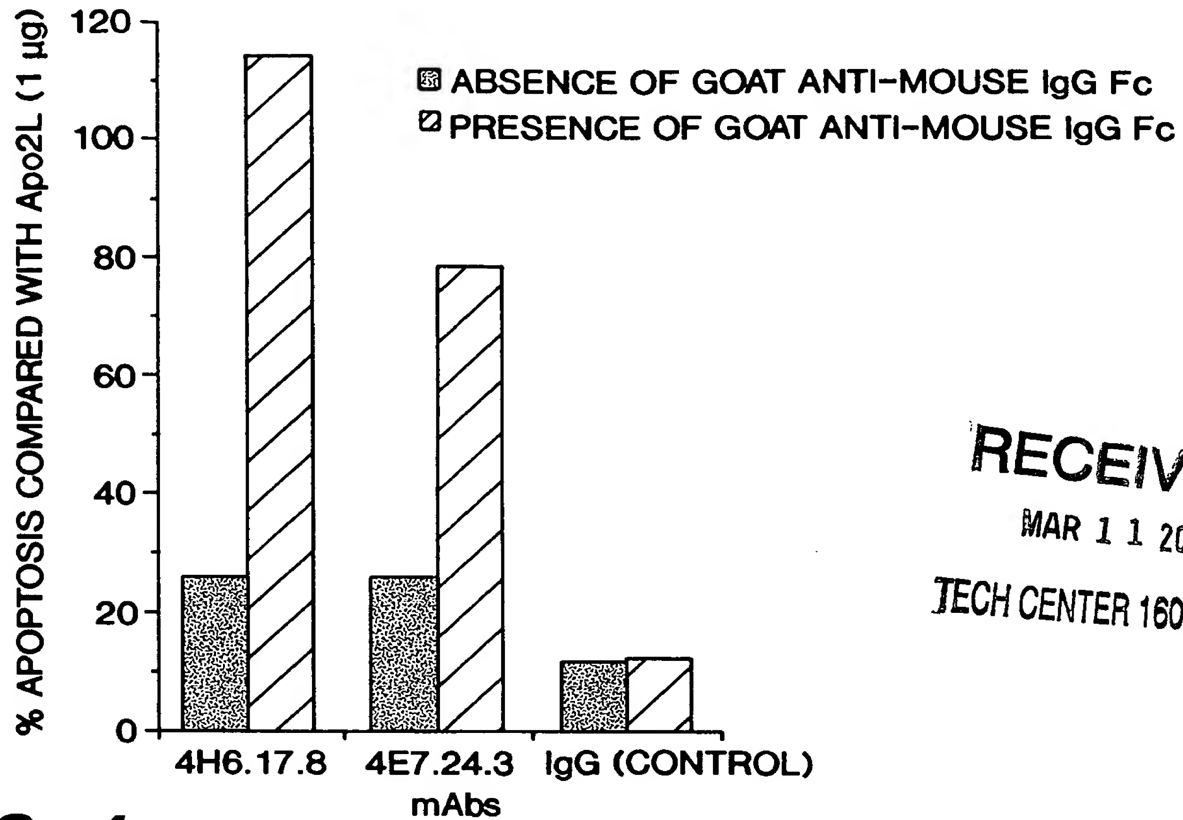

% APOPTOSIS COMPARED WITH Apo2L (1 μg)
120
100
80
60
40
20
0
ABSENCE OF GOAT ANTI-MOUSE IgG Fc
PRESENCE OF GOAT ANTI-MOUSE IgG Fc
4H6.17.8
4E7.24.3
IgG (CONTROL)
mAbs

FIG._4

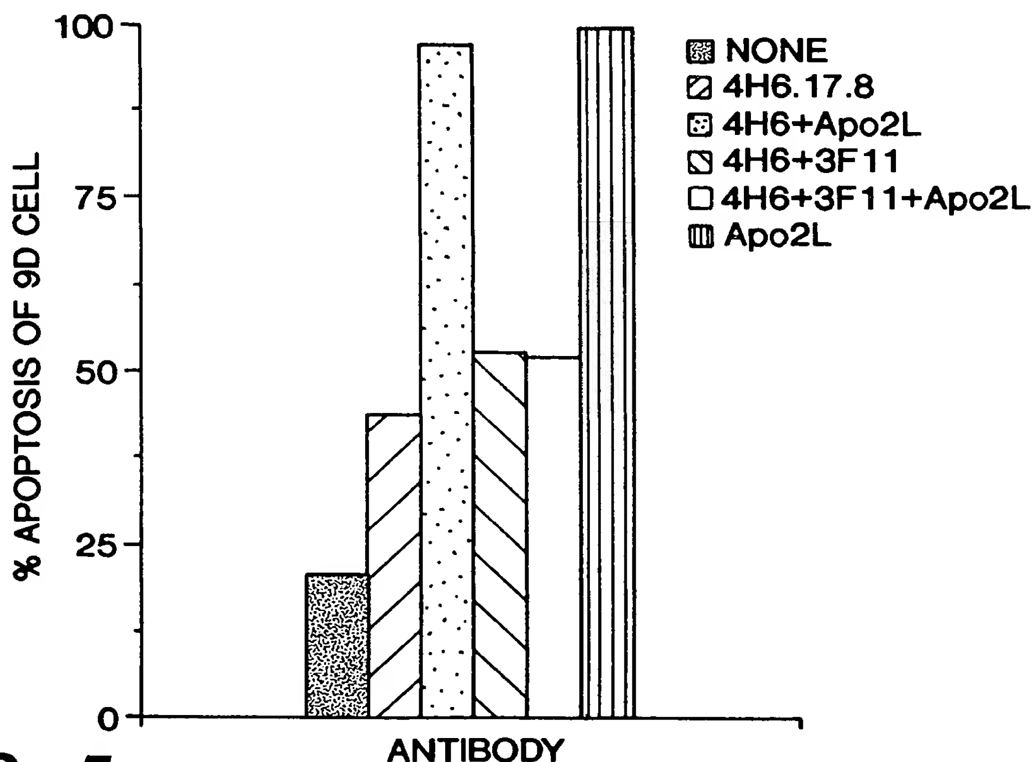

% APOPTOSIS OF 9D CELL
100
75
50
25
0
NONE
4H6.17.8
4H6+Apo2L
4H6+3F11
4H6+3F11+Apo2L
Apo2L
ANTIBODY

FIG._5

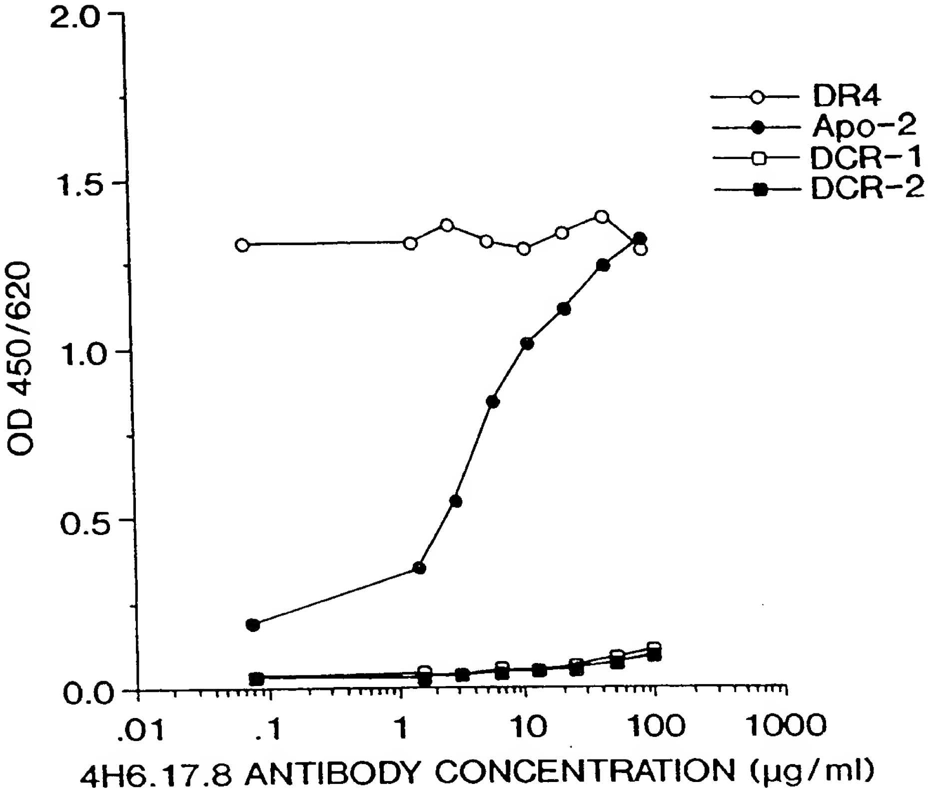

2.0
1.5
1.0
0.5
0.0
OD 450/620
DR4
Apo-2
DCR-1
DCR-2
.01
.1
1
10
100
1000
4H6.17.8 ANTIBODY CONCENTRATION (μg/ml)

FIG. 6A

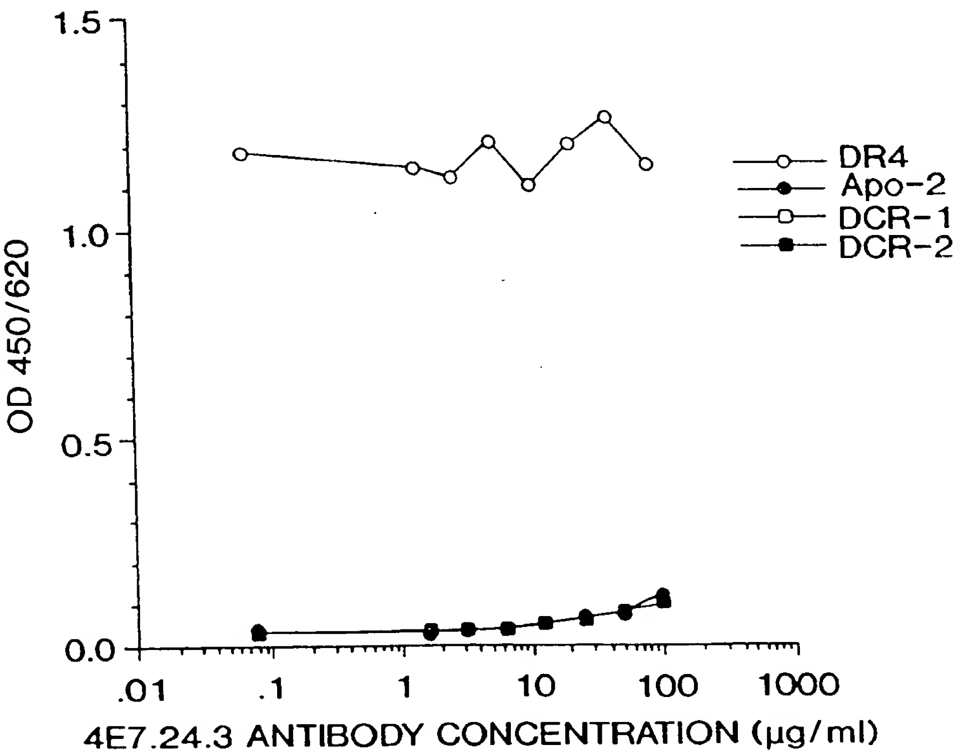
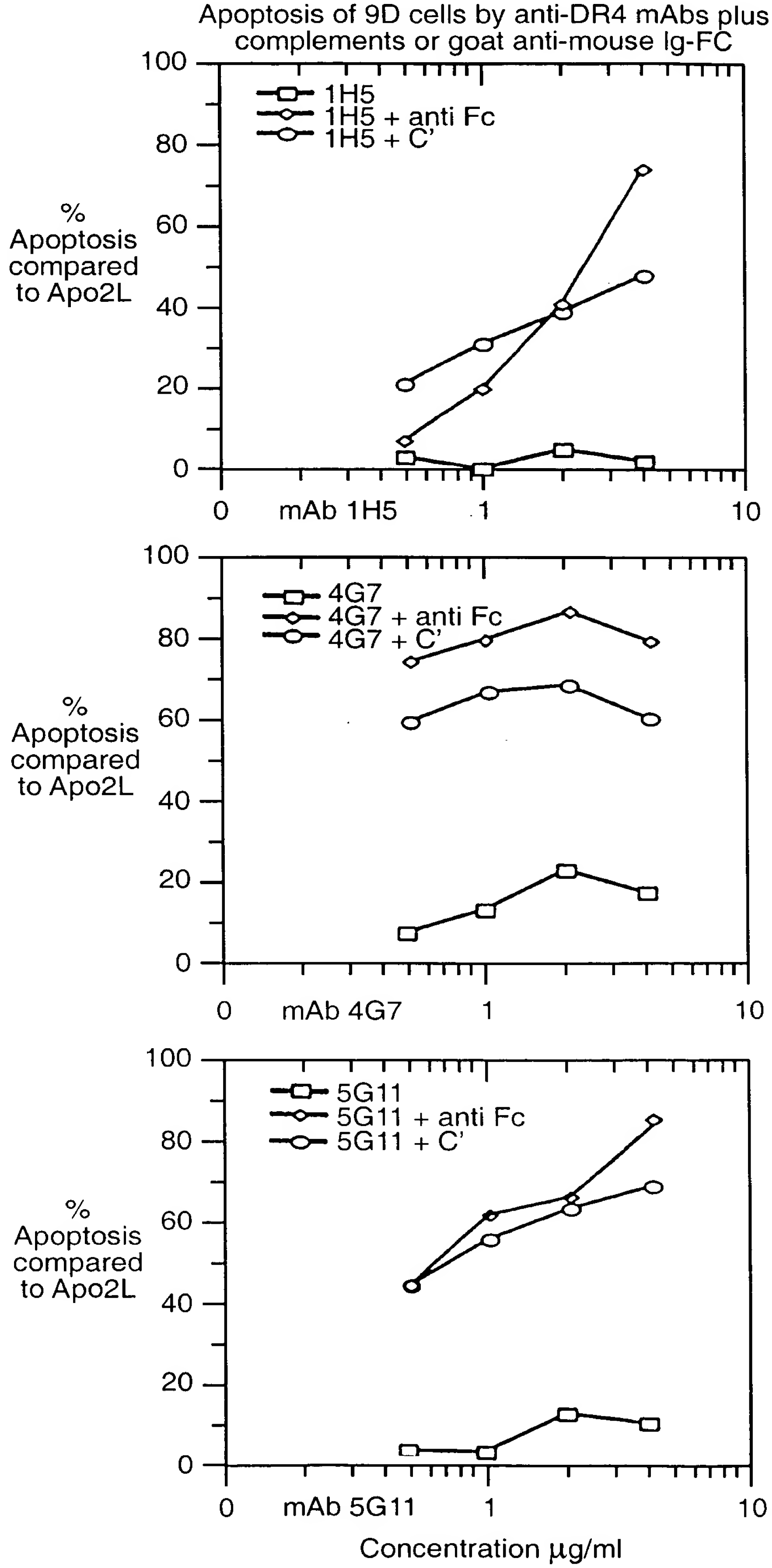

1.5
1.0
0.5
0.0
OD 450/620
DR4
Apo-2
DCR-1
DCR-2
.01
.1
1
10
100
1000
4E7.24.3 ANTIBODY CONCENTRATION (μg/ml)

FIG. 6B

FIG._8A


Apoptosis of 9D cells by anti-DR4 mAbs plus complements or goat anti-mouse Ig-FC
1H5
1H5 + anti Fc
1H5 + C'
% Apoptosis compared to Apo2L
100
80
60
40
20
0
0
mAb 1H5
1
10
4G7
4G7 + anti Fc
4G7 + C'
% Apoptosis compared to Apo2L
mAb 4G7
5G11
5G11 + anti Fc
5G11 + C'
% Apoptosis compared to Apo2L
mAb 5G11
Concentration μg/ml

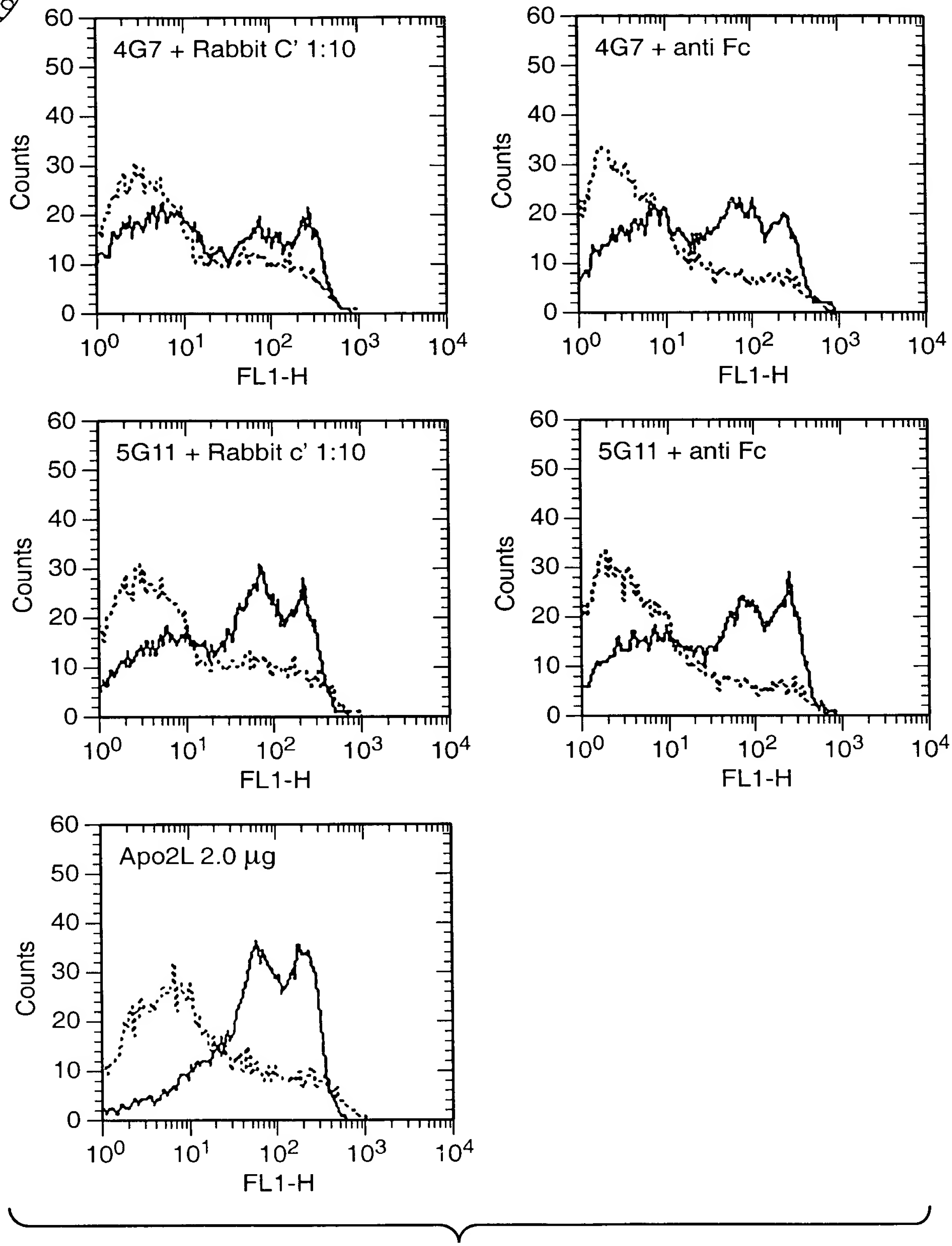

4G7 + Rabbit C' 1:10
4G7 + anti Fc
5G11 + Rabbit c' 1:10
5G11 + anti Fc
Apo2L 2.0 μg
Counts
FL1-H
0
10
20
30
40
50
60
10^0
10^1
10^2
10^3
10^4

FIG._8B

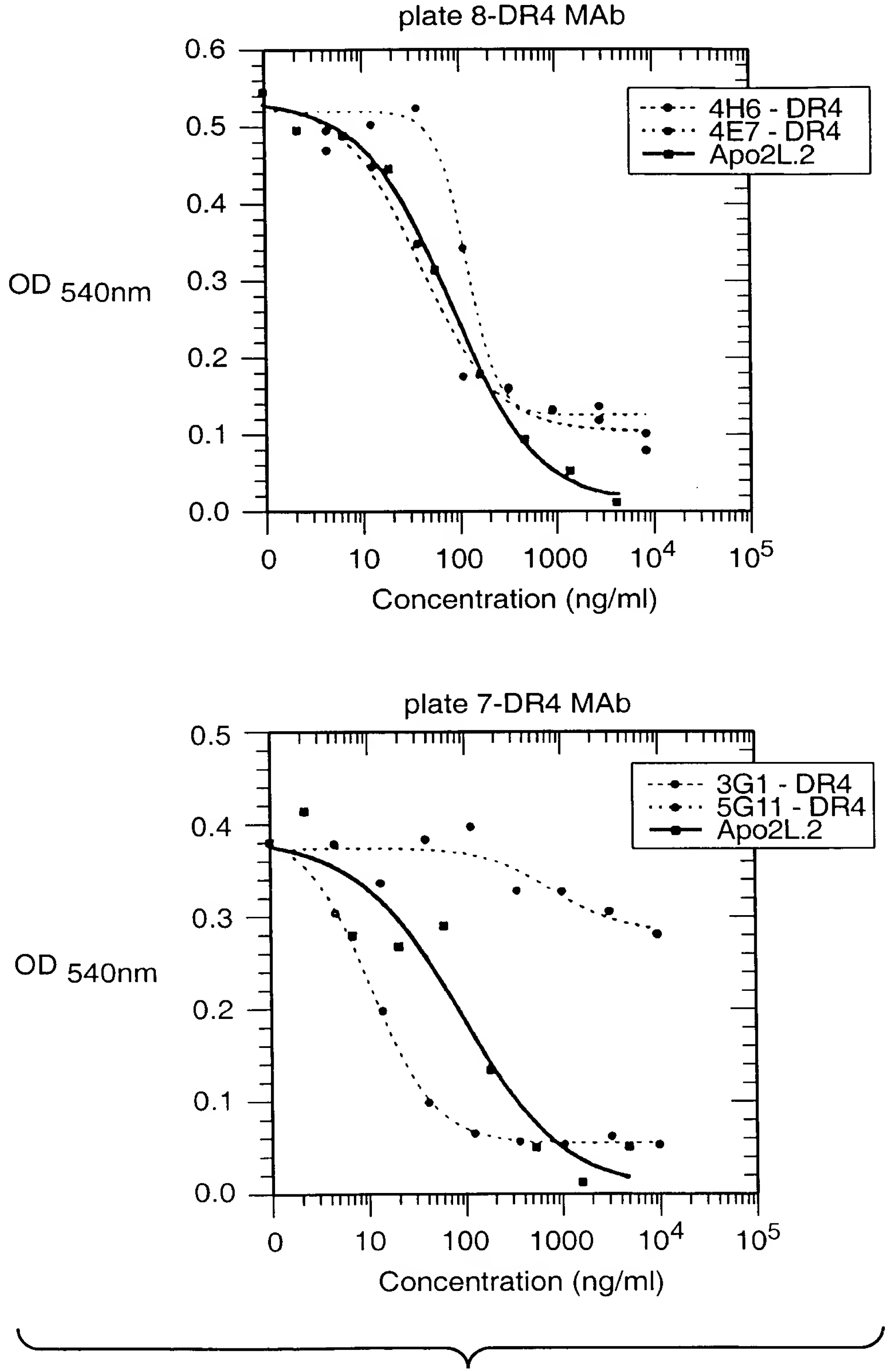


plate 8-DR4 MAb
4H6 - DR4
4E7 - DR4
Apo2L.2
OD 540nm
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
10
100
1000
10^4
10^5
Concentration (ng/ml)
plate 7-DR4 MAb
3G1 - DR4
5G11 - DR4
Apo2L.2
OD 540nm
0.5
0.4
0.3
0.2
0.1
0.0
0
10
100
1000
10^4
10^5
Concentration (ng/ml)

FIG._9A

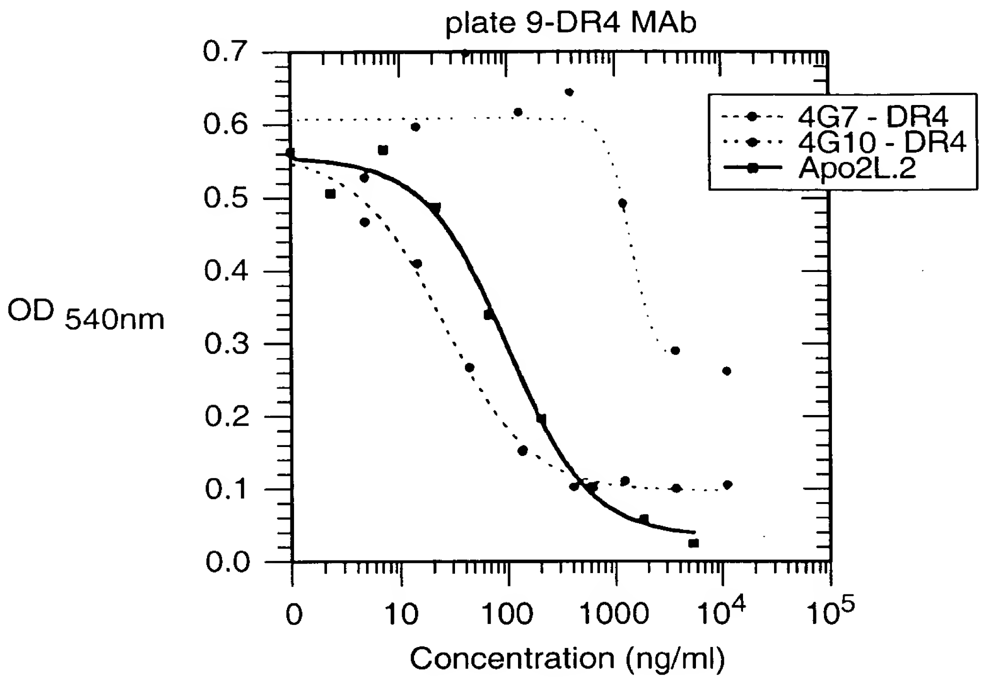


plate 9-DR4 MAb
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
OD 540nm
4G7 - DR4
4G10 - DR4
Apo2L.2
0
10
100
1000
10^4
10^5
Concentration (ng/ml)

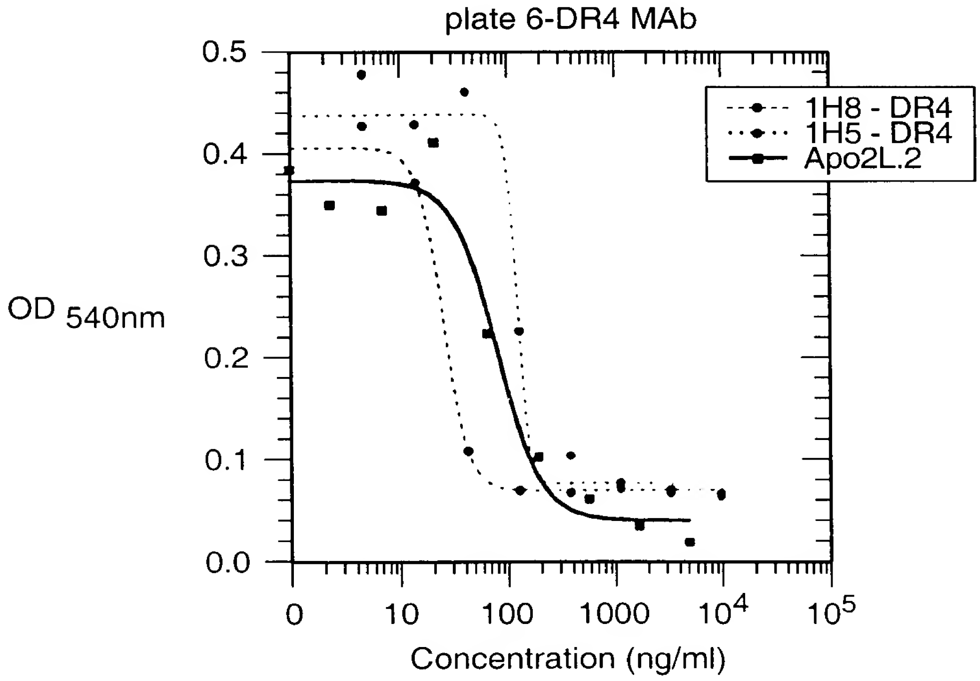


plate 6-DR4 MAb
0.5
0.4
0.3
0.2
0.1
0.0
OD 540nm
1H8 - DR4
1H5 - DR4
Apo2L.2
0
10
100
1000
10^4
10^5
Concentration (ng/ml)

FIG._9B

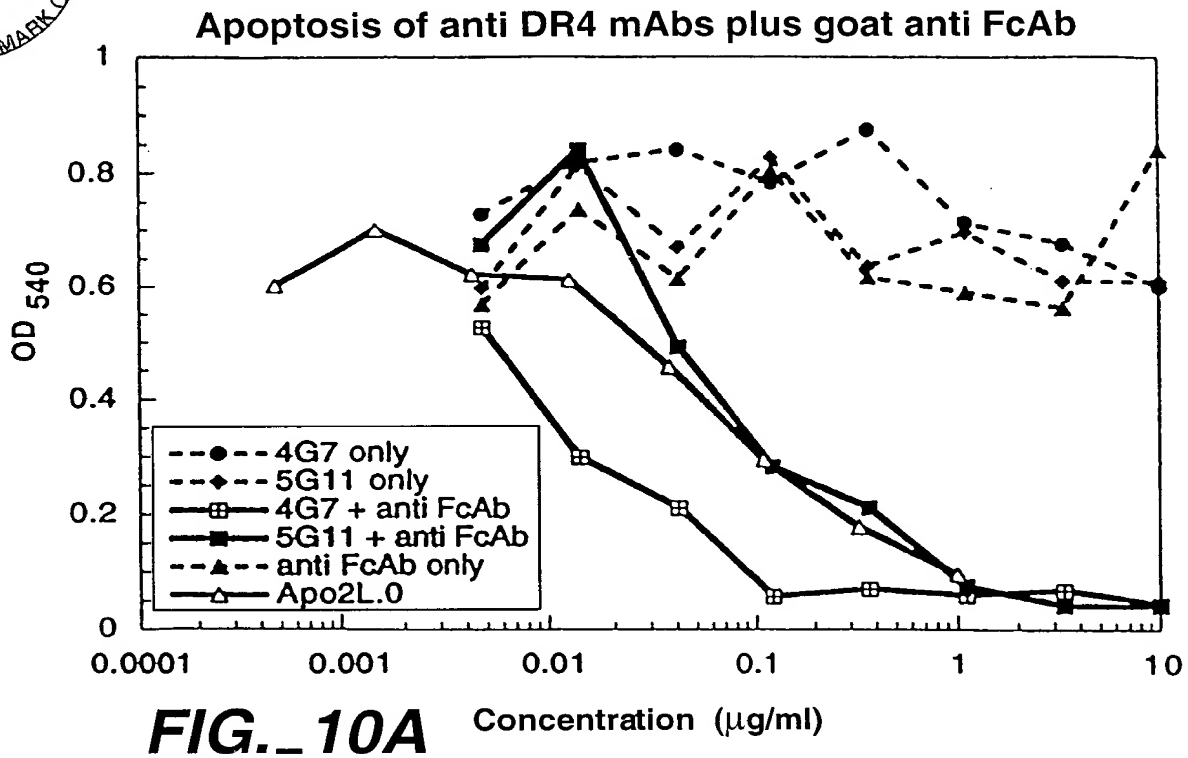


FIG._10A

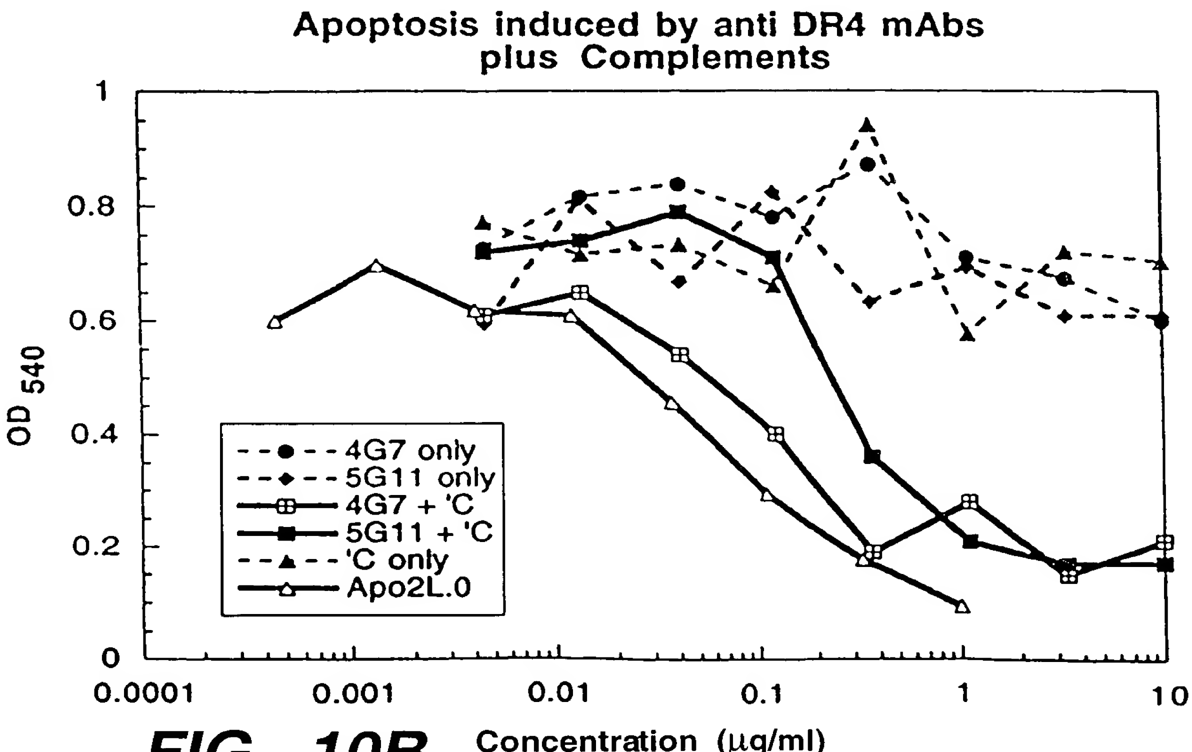


FIG._10B

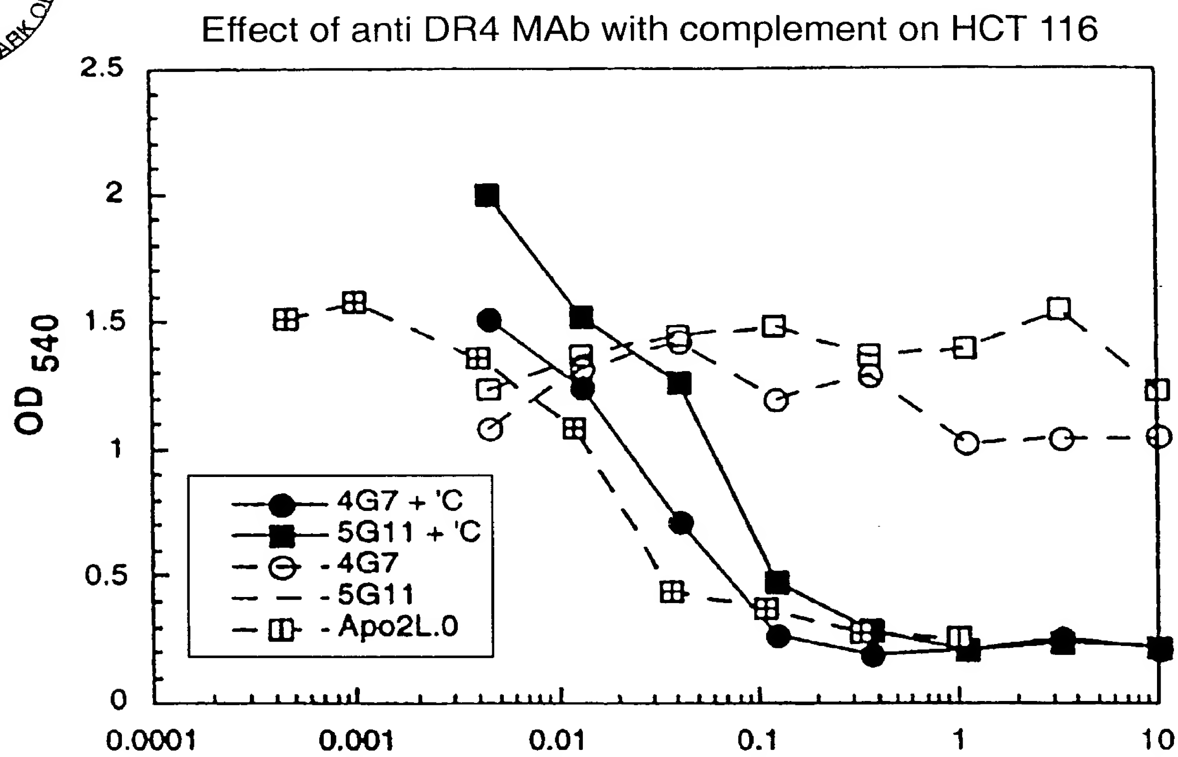


FIG._11A

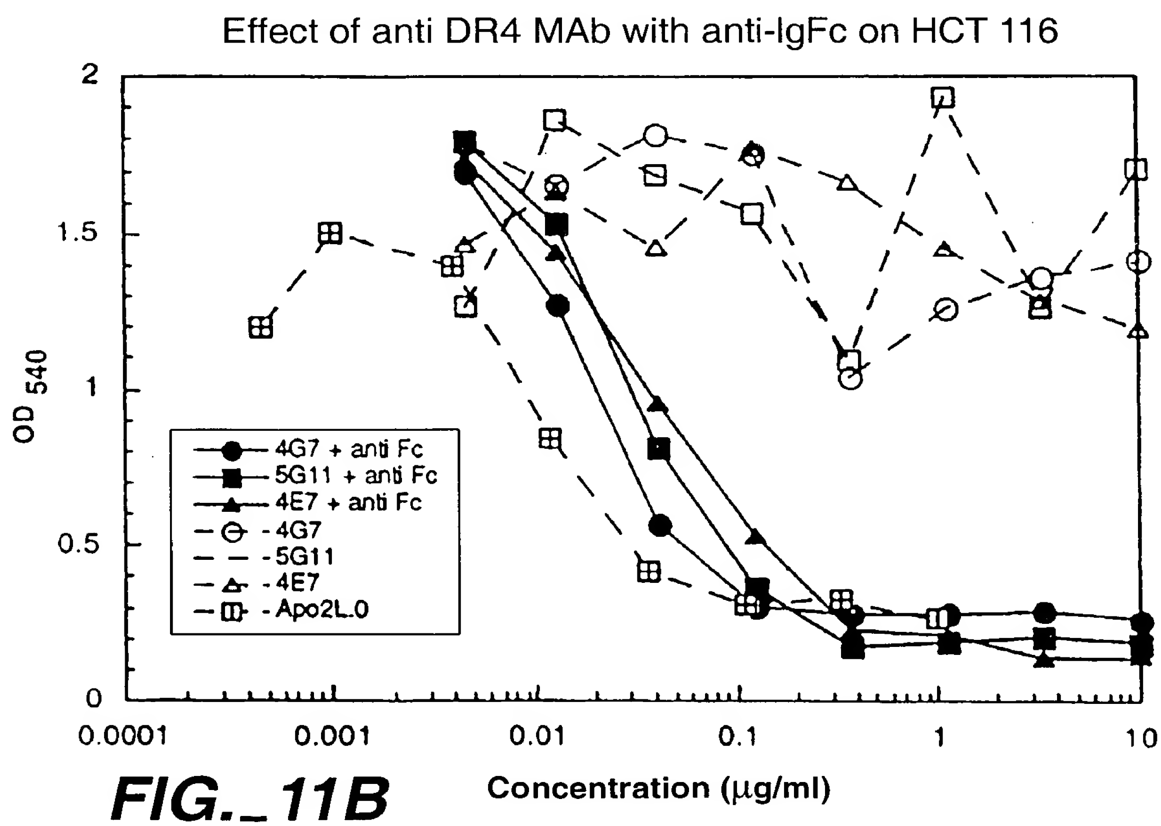


FIG._11B

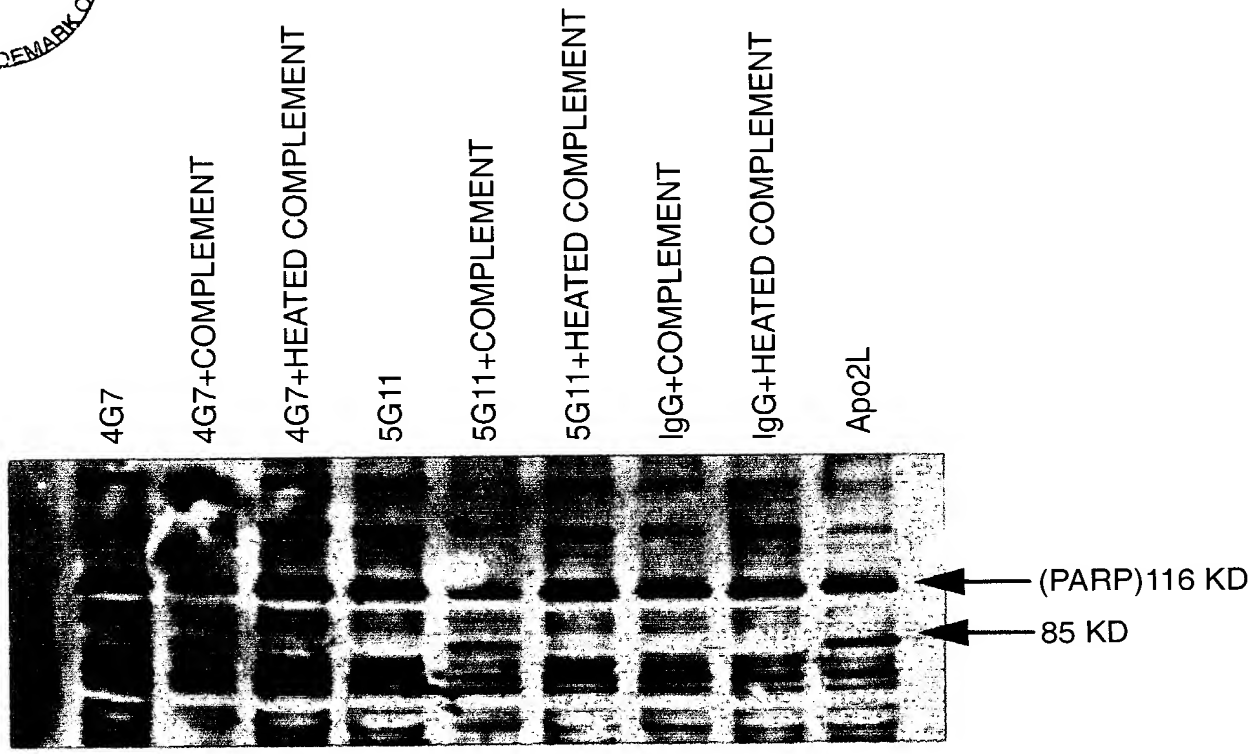

4G7
4G7+COMPLEMENT
4G7+HEATED COMPLEMENT
5G11
5G11+COMPLEMENT
5G11+HEATED COMPLEMENT
IgG+COMPLEMENT
IgG+HEATED COMPLEMENT
Apo2L
(PARP)116 KD
85 KD

FIG._12

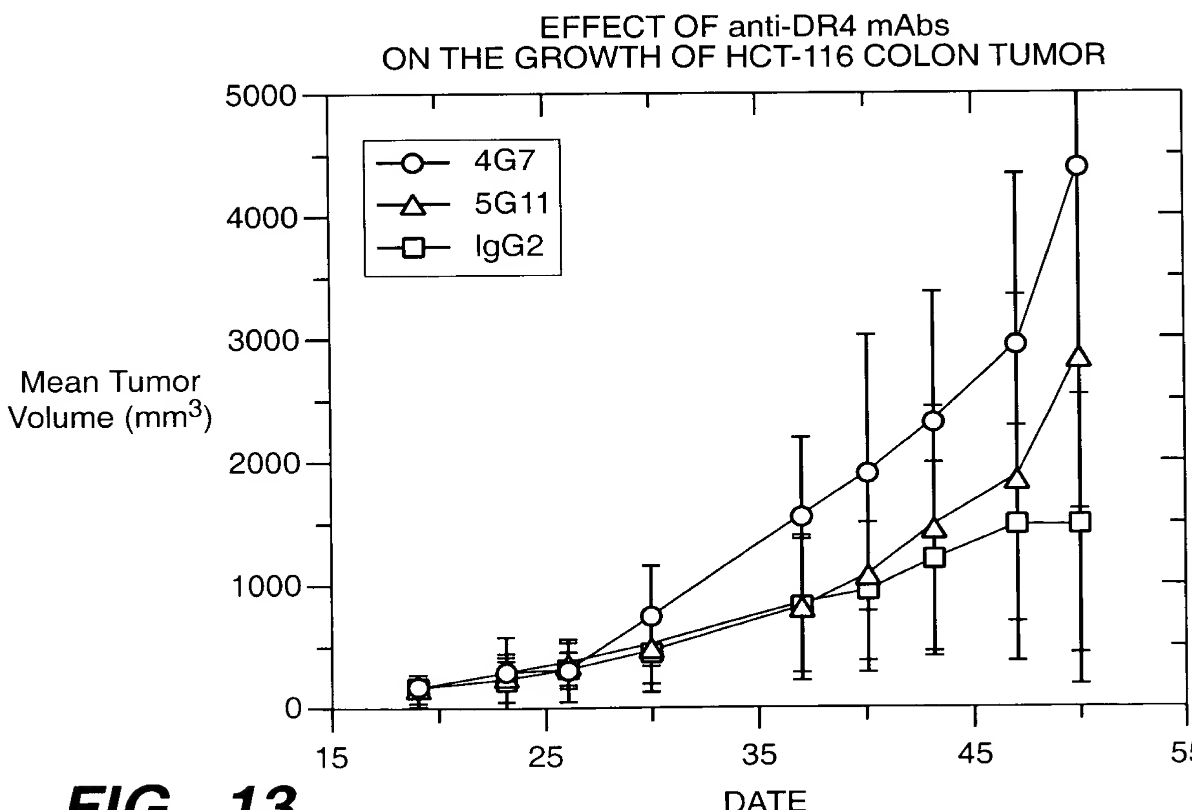

EFFECT OF anti-DR4 mAbs
ON THE GROWTH OF HCT-116 COLON TUMOR
Mean Tumor Volume (mm3)
DATE
4G7
5G11
IgG2
5000
4000
3000
2000
1000
0
15
25
35
45
55

FIG._13

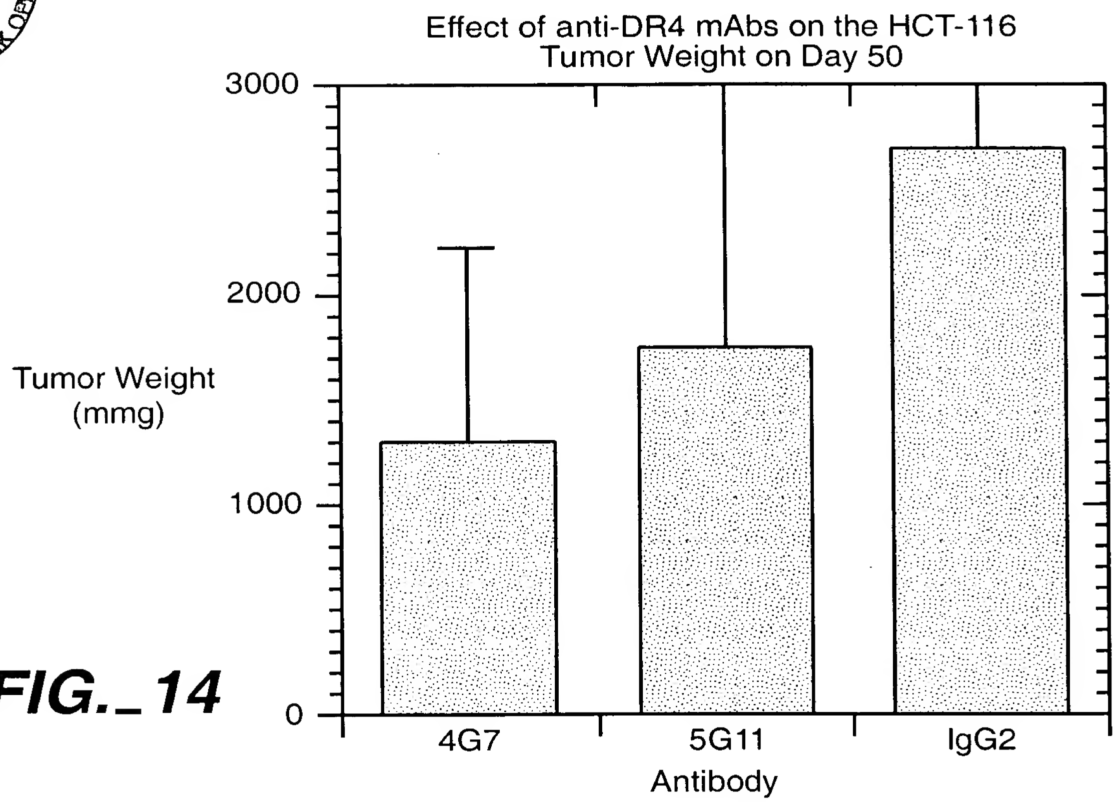

Effect of anti-DR4 mAbs on the HCT-116
Tumor Weight on Day 50
Tumor Weight (mmg)
3000
2000
1000
0
4G7
5G11
IgG2
Antibody

FIG._14

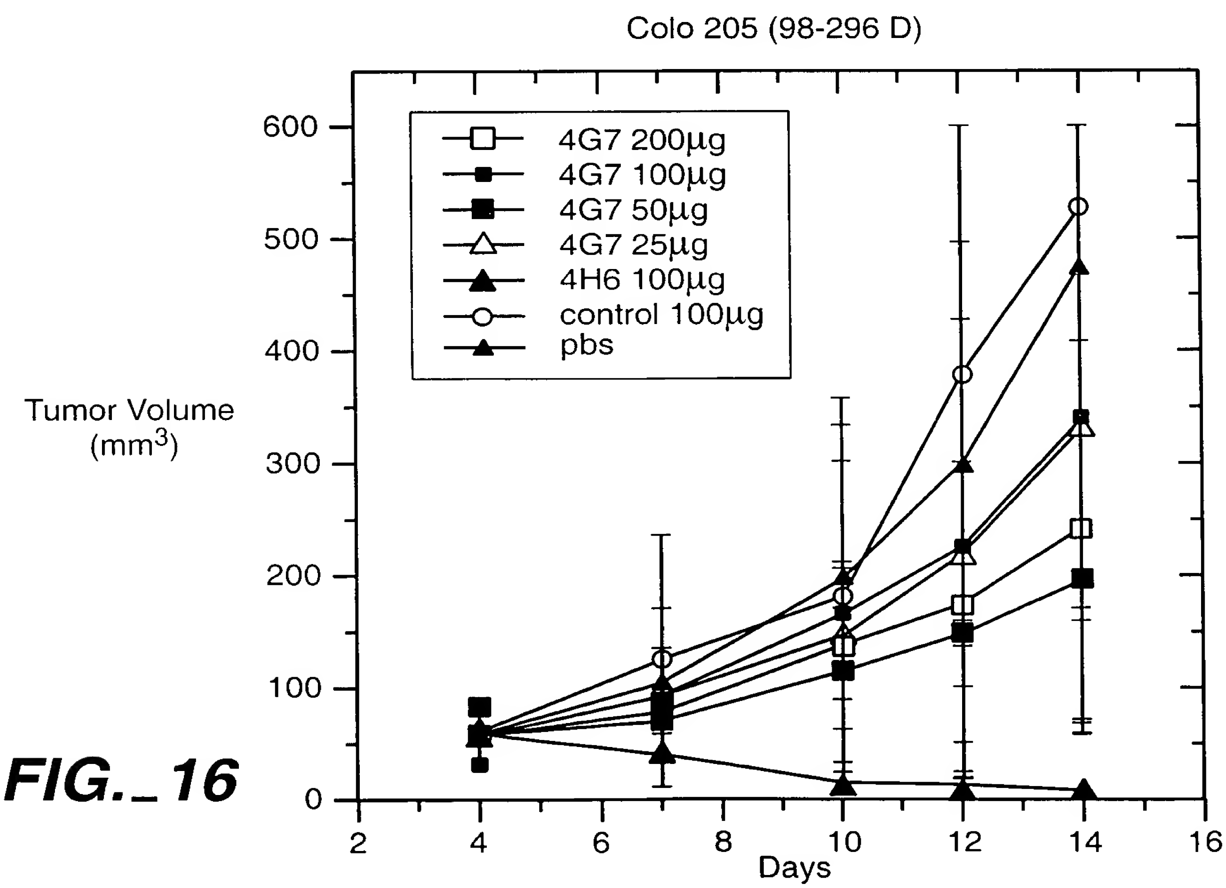

Colo 205 (98-296 D)
Tumor Volume (mm^3)
600
500
400
300
200
100
0
4G7 200μg
4G7 100μg
4G7 50μg
4G7 25μg
4H6 100μg
control 100μg
pbs
2
4
6
8
10
12
14
16
Days

FIG._16

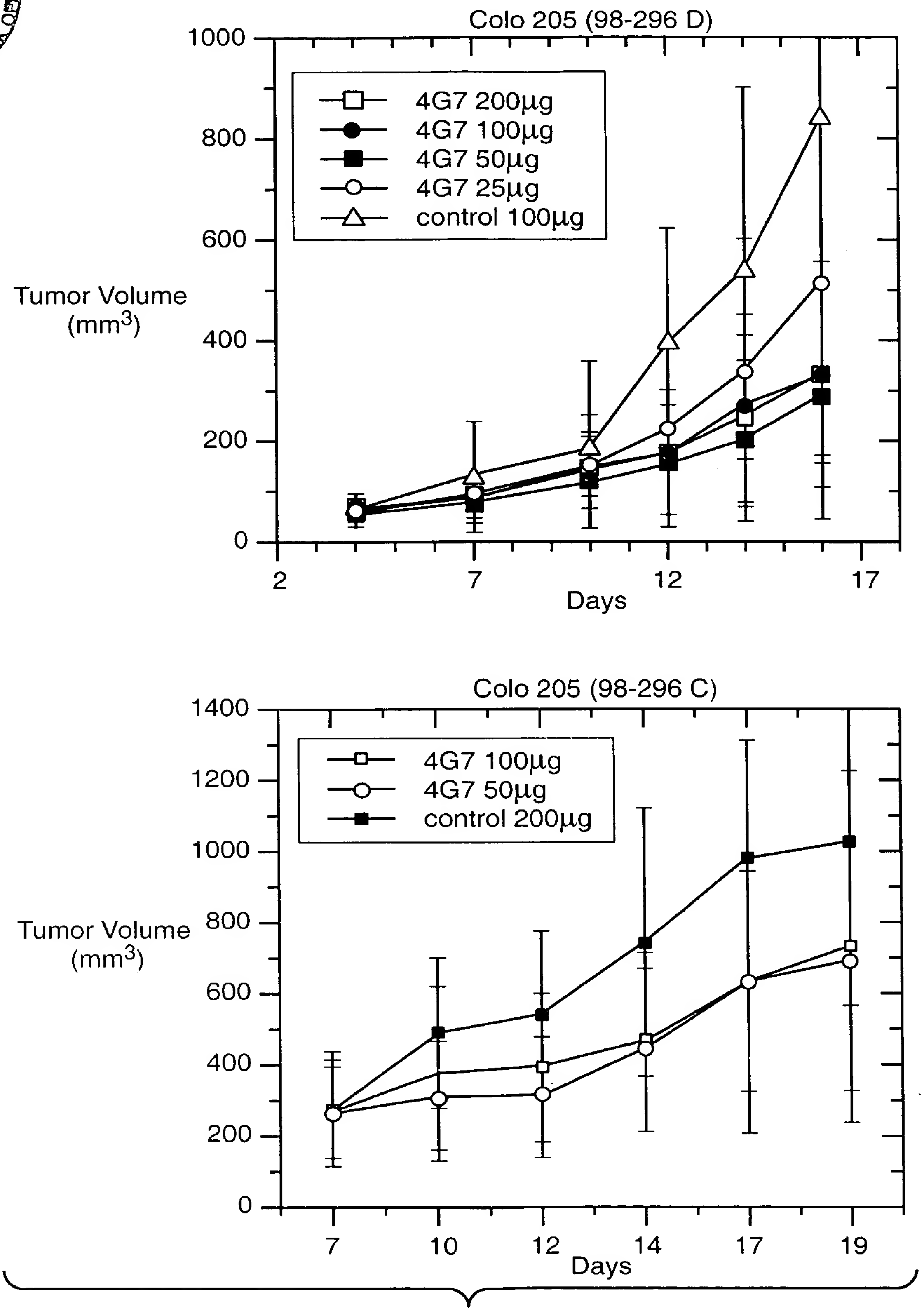

Colo 205 (98-296 D)
4G7 200µg
4G7 100µg
4G7 50µg
4G7 25µg
control 100µg
Tumor Volume (mm3)
Days
0
200
400
600
800
1000
2
7
12
17
Colo 205 (98-296 C)
4G7 100µg
4G7 50µg
control 200µg
Tumor Volume (mm3)
Days
0
200
400
600
800
1000
1200
1400
7
10
12
14
17
19

FIG._15

GENERAL CHARACTERISTICS OF ANTI-DR4 mAbs

	Isotype	Kd-1 (pm)	Apop w / o L	Apop + &Fc	Apop + C'	Block	Cross Reactivity DR4	DR5	DCR1	DCR2
1H5.24.9	IgG2a			-	-	ND	+++	-	-	-
1H8.17.5	IgG1			+	ND	ND	+++	-	-	-
3G1.17.2	IgG1			-	ND	-	+++	-	-	-
4E7.24.3	IgG1	2	+ / -	+	-	-	+++	+	-	+ / -
4G7.18.8	IgG2a		+ / -	+	+	-	+++	-	-	-
4H6.17.8	IgG1	5	+ / -	+	-	+	+++	+	-	-
4G10.20.6	IgG1			+	ND	-	+++	+	-	-
5G11.17.1	IgG2b	22		+	+	ND	+++	++	-	-

All these mAbs recognize DR4 on 9D cells and immune precipitate DR4-IgG.

w / o L: The apoptotic ability of mAbs by themselves was detected on 9D cells, skmes cells, HCT116 and colo 205

+ &FC: The apoptotic ability of mAbs was determined in combination with goat anti-mouse IgG FC.

+ C': The apoptotic ability of mAbs was determined in the presence of rabbit complement

Degrees of binding (+) to DR5 by Mabs 4E7 and 4H6 at 10 μg / ml are 15% of the binding of DR4.

FIG._17